Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

The role of biopesticides in sustainable agriculture

Nature fighting nature

Susan M. Boyetchko

Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan,Canada

Sue.Boyetchko@Canada.ca

Canada

Introduction

- Investment in biopesticide R&D in Canada has progressed
- perceptions and attitudes towards chemical pesticides have changed
- renewed interest in biopesticides, more products being registered since 2000
- social and economic drivers
  - legislative changes
  - regulatory policies
  - changing attitudes of consumers
  - greater interest by small-to-medium sized enterprises (SME's)

What are biopesticides?

- beneficial use of living organisms to (directly or indirectly) suppress, inhibit, damage, or kill a pest or pest population
- biocontrol agents: e.g. fungi, bacteria, viruses, natural products
- inundative application, applied repeatedly, annually
- easy to use and mass-produce, acceptable shelf life
- host specific (target pests/pathogens, group of related pathogens)
- no detrimental effects on non-target organisms
- environmental and toxicological safety standards

Biopesticides – Opportunities/Need

- pesticide-resistance management
- control of invasive alien species
- reduced risk pest control products (new active ingredients & new modes of action)
- expand label registration of existing biopesticide products; more products registered in Canada
- reduce chemical residues (soil, water, food)
- IPM in crop production systems (e.g. conventional, organic, no/low pesticide use)
- where control measures (e.g. chemicals) are inadequate/unavailable/deregistered

Biopesticides = Next Generation of Pest Control Products

(transformative research)

Biopesticide Market

Global Biopesticides and Synthetic Pesticides Market ($millions)

Type	2003	2004	2005	2010	Ave. Ann. growth rate
Biopesticides	468	562	672	**1,075**	**9.9**
Synthetic Pesticides	27,144	26,600	26,076	24,205	-1.5
Total	27,612	27,162	26,748	25,280	-1.1
Biopesticides as % of total	1.69	2.07	2.51	4.25	

from Business Communications Company, Inc. (www.bccresearch.com)

Growth in the biopesticide market is expected to be 10X greater than for synthetic pesticides.

THE BIOPESTICIDE MARKET

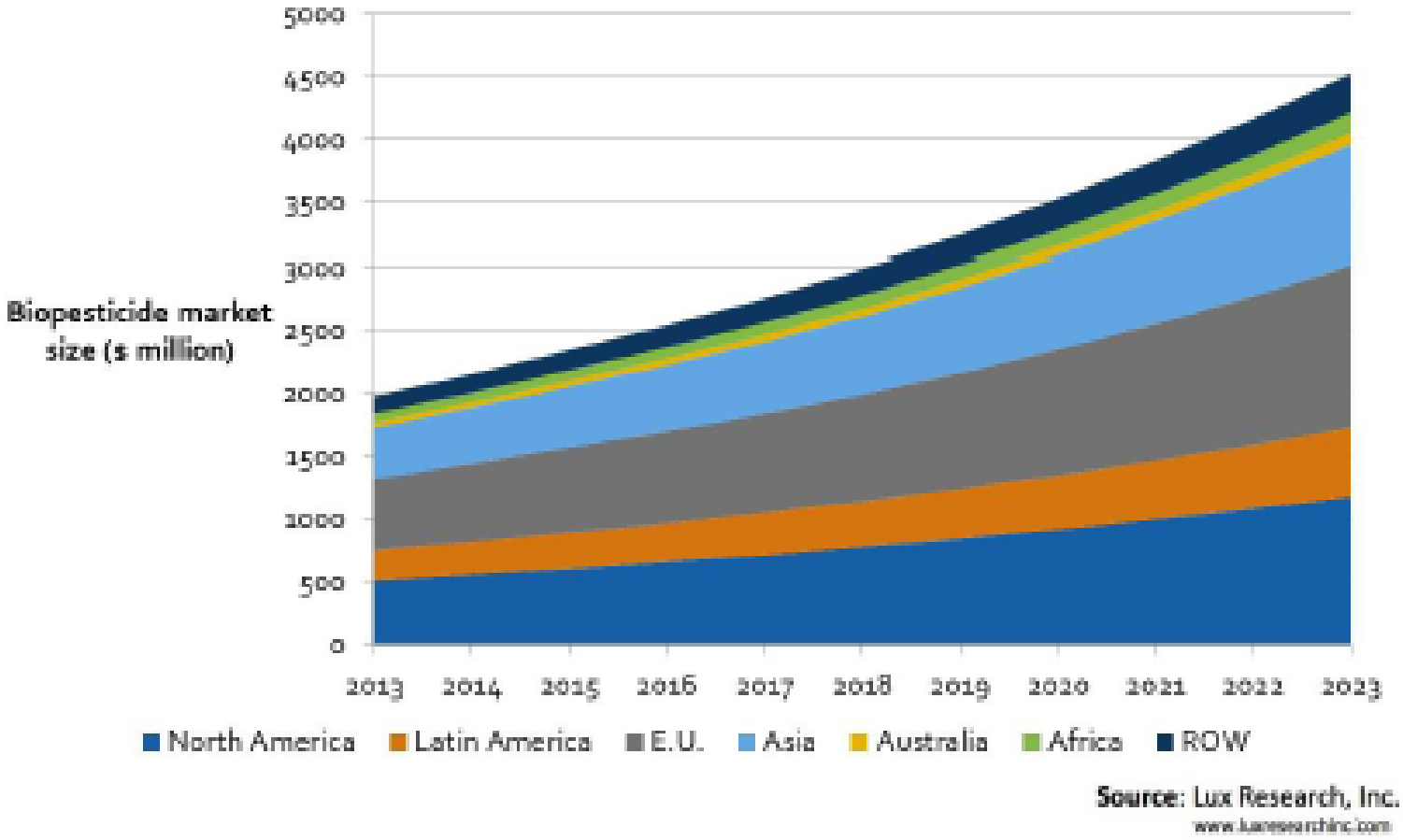


Figure 1. The biopesticide market is approximately $3 billion today, and will rise above $4.5 billion by 2023.

Olson 2015

Perceptions of biopesticides in comparison to chemicals

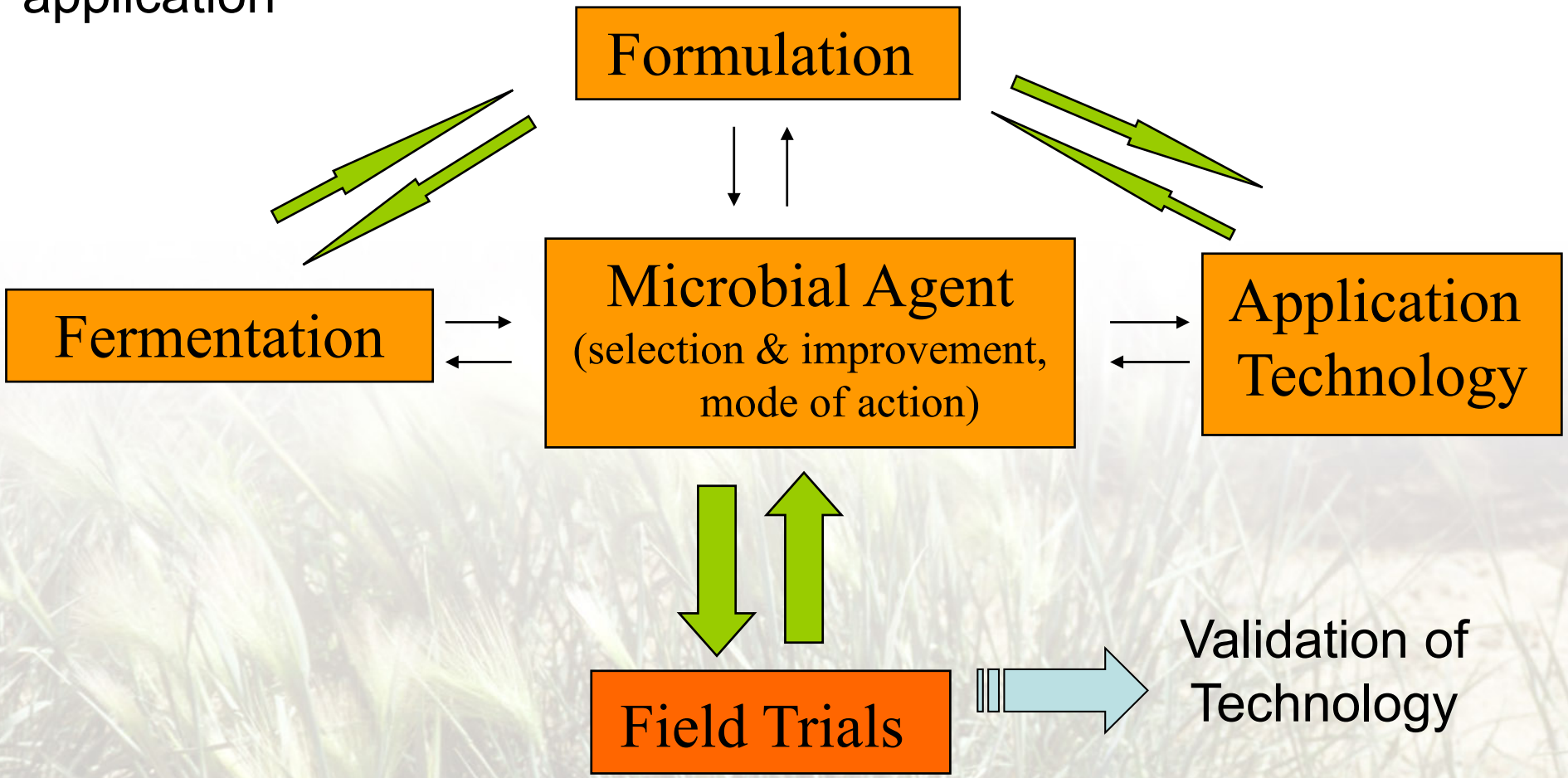
- biopesticides have lower efficacy
- pest control not as rapid as chemicals
- narrow spectrum of activity with biologicals (market size)
- how to expand spectra of activity
- biopesticides are more difficult to use
- chemicals provide “silver-bullet” approach/one-time use
- consumers/producers are risk-averse and have few reasons or incentives to change pest control practices
- biased comparisons are made between biologicals and chemicals
- agricultural crops and home-gardens must be pristine and weed-free

How do we change these perceptions and improve adoption of biopesticides?

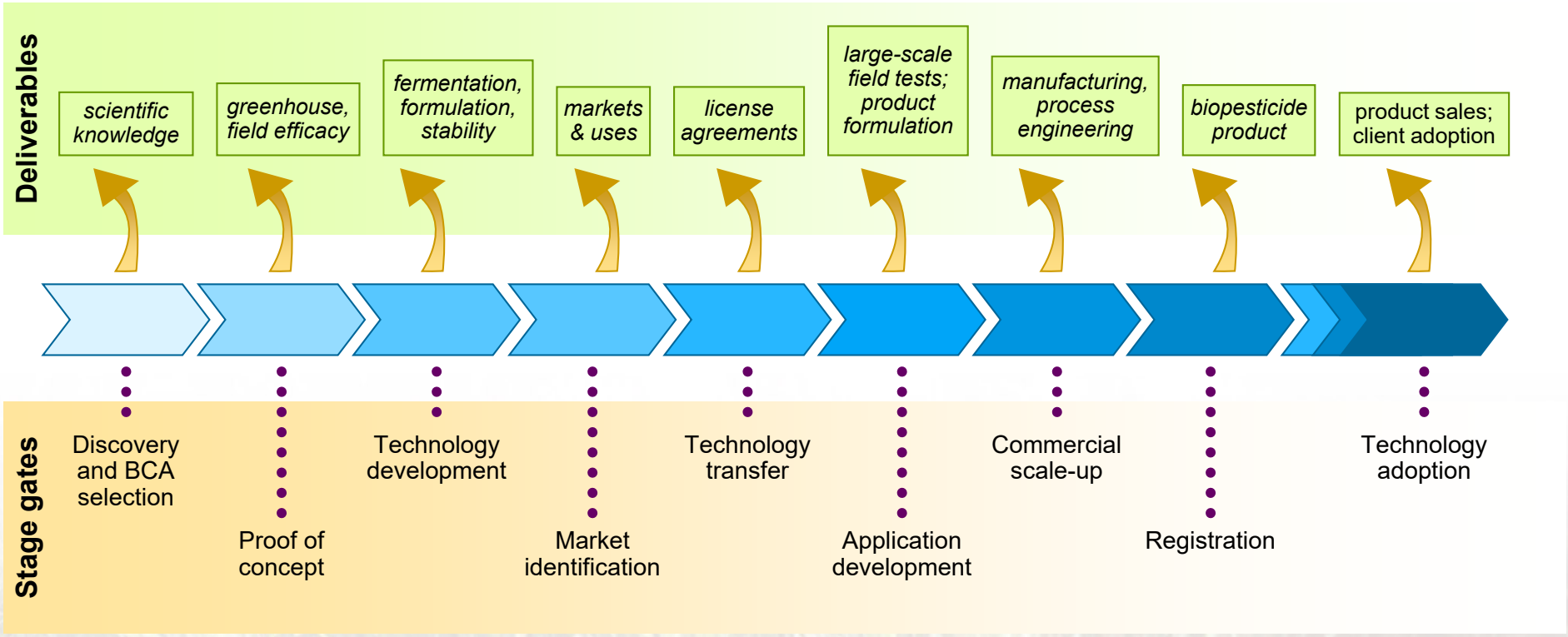
- Invest in Research & Development
  - build research expertise, capacity
  - assemble multidisciplinary teams
- Establish Critical Mass
  - develop effective partnerships between government, academia, and industry
  - expertise in microbiology, plant pathology, weed science, agronomy, chemistry, engineering
- Create Education and Communication Programs
  - public awareness and outreach programs
  - general public, producers, extension personnel, industry
  - early education in schools and universities

Product Development – Strategy

Platform technologies:
fermentation, formulation, spray application



Biopesticides – Science Innovation Chain
Our Road Map



Regulatory process

Basic science and concept design

Advanced knowledge

Applied research

Greater Investment

- Biopesticide industry becoming better organized (e.g. BPIA); meet with key regulators
- Acquisition of biopesticide SME's by multi-nationals
  - BASF → Becker Underwood
  - Bayer CropScience → AgraQuest & Prophyta
  - Syngenta → Pasteuria BioScience
  - Monsanto → Novozymes
- Licensing technology from government and university scientists

It starts with fermentation…

Solid-state

Liquid-state

Biphasic systems

Formulation Criteria

- Compatible with biopesticide; not phytotoxic to crop
- Facilitate dispersal, deposition and retention
- Promote efficacy on the target
- Protect from shear forces during spray
- Protect against heat, desiccation, and UV radiation
- Stability during production and storage
- Amenable for commercial packaging & handling
  Apply through conventional farming equipment.
- Improve safety to users and environment

- ✓ **Composition:**
  - **Active ingredient** – biological agent, metabolites
  - **Carriers** – clay, peat, oil, grain, inerts, biofilms, water (separate or integrated with biological)
  - **Adjuvants** – humectants, dispersants, stickers, spreaders, binders, surfactants, synergists, nutrients, sunscreens
- ✓ **Type:**
  - **Granules:** made from solid or liquid fermentations
  - **Wettable powders:** dehydrated liquids-spray dryers
  - **Oil-based emulsions:** water-oil-water (WOW)
  - **Micro-encapsulation** of solids or liquids

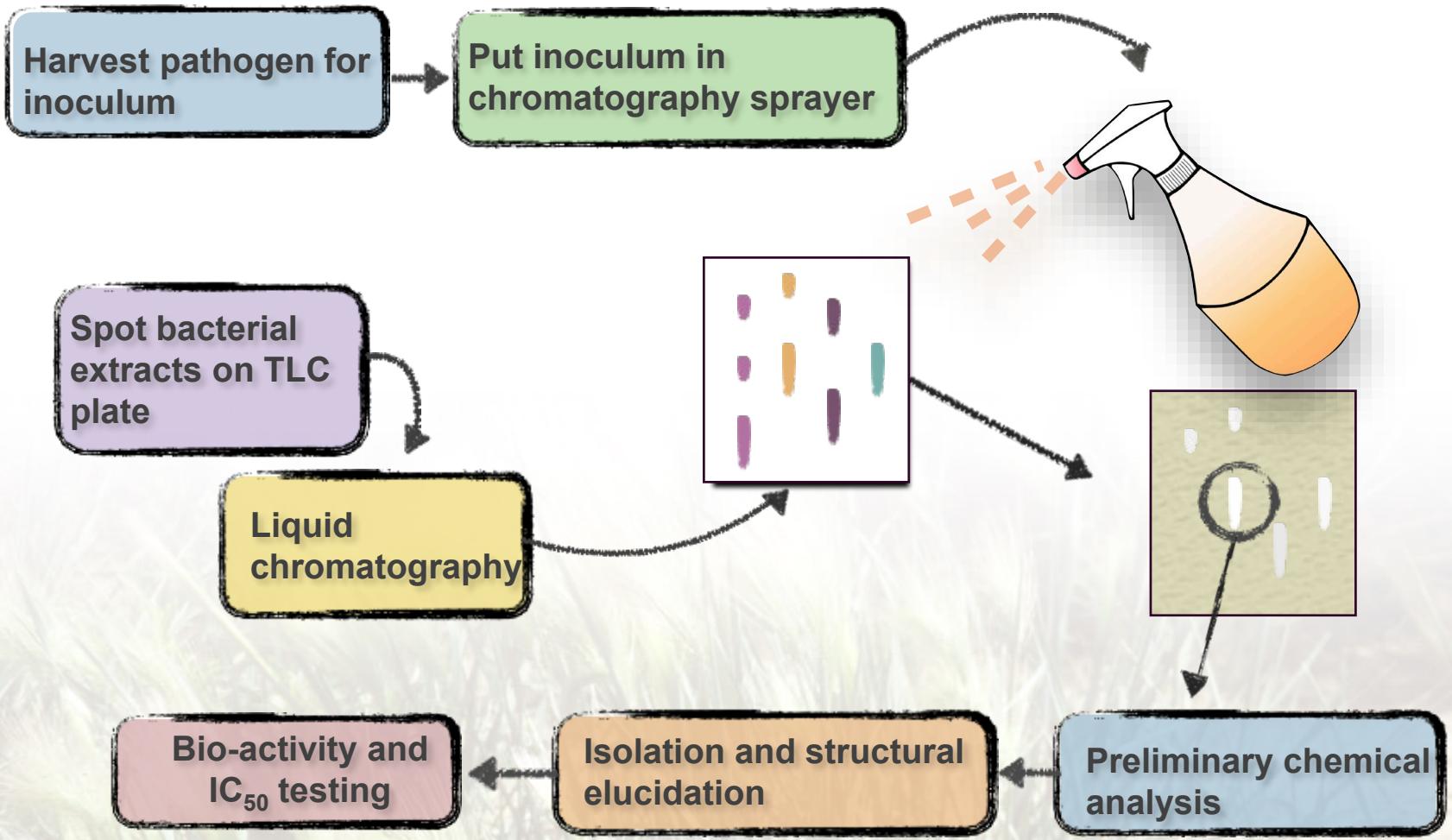
Endophytes

- Fungi or bacteria that occur and develop inside plant tissues without causing harm
- Microbes with biocontrol properties
- Overcome delivery/application issues associated with biopesticides
- Cheaply introduced into seeds, tissue culture plantlets
- Provide protection against adverse environment conditions
- Possess additional beneficial properties (accelerate plant emergence), plant growth promotion

Natural products

- Sophisticated technology: bioassay directed; NMR, UPLC-MS, GC
- Use of databases to determine if known or unknown natural products

Natural Products: Direct Bioautography



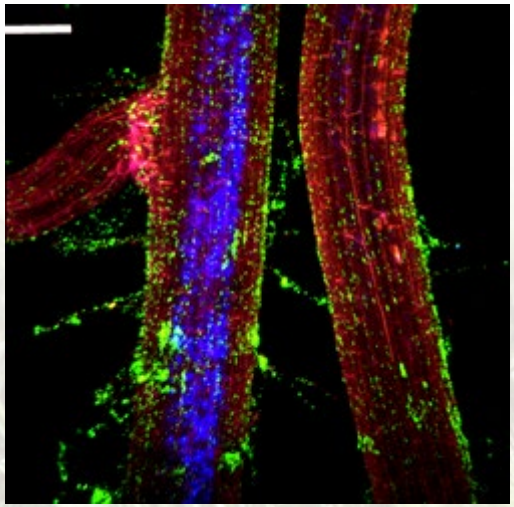
Research Projects

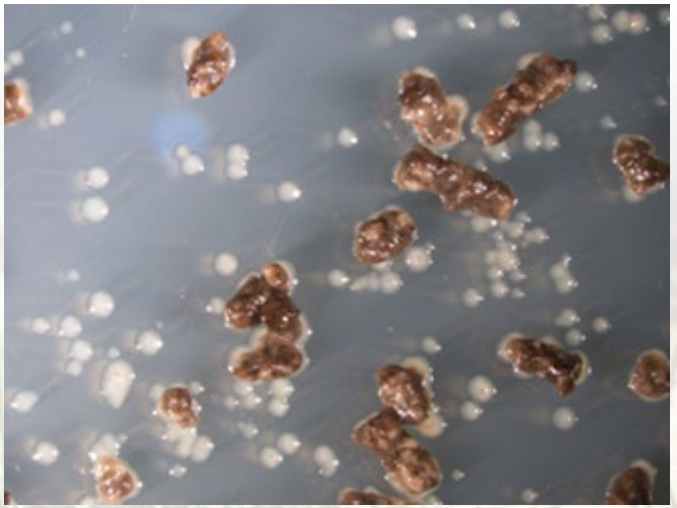
- Soil bacteria as a pre-emergent bioherbicide (Susan Boyetchko, Russell Hynes)
- Waging a war on potato late blight: a biological solution for a global disease (Susan Boyetchko, Patrice Audy, Tim Dumonceaux, Chris Kirby, Ting Zhou)
- Biopesticides as a Novel Management Strategy for Sclerotinia in Canola (Susan Boyetchko, Chrystel Olivier, Fengqun Yu, Tim Dumonceaux, Abdulsalam Dakouri, Chris Kirby)

Pre-emergent soil bacteria as bioherbicides for annual grass weeds - *Pseudomonas fluorescens* strain BRG100

- Wild oat and green foxtail
  - 2 most important grass weeds in Canadian prairies/Great Plains region
  - Soil bacteria applied as pre-emergent bioherbicides
  - large-scale field production

Canadian and US patents issued





Susan Boyetchko & Russell Hynes

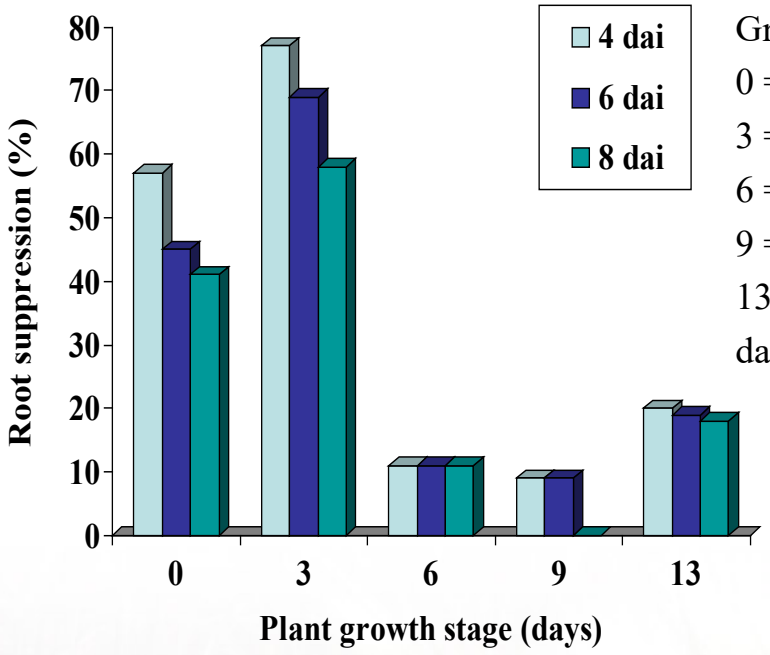
Pseudomonas fluorescens strain BRG100
Annual grass weed control

- early proof-of-concept
- field validation
- mini-plots (1 or 2 m^2 plots)
- seeded weeds by hand; pre-determined weed densities

Green Foxtail - untreated

Green Foxtail - BRG100

Soil bacteria – growth stage



Growth stages:
0 = germination
3 = 1 leaf stage
6 = 1-2 leaf stage
9 = 2 leaf stage
13 = 2-3 leaf stage
dai = days after inoculation

- susceptibility at germination to early root growth
- no effect as a foliar spray or beyond 1-2 leaf stage
- **pre-emergent application**
- **Broad spectrum activity on other grass weed species**

Pseudophomin A ($C_{55}H_{98}N_9O_{16}$);
Pseudophomin B ($C_{57}H_{102}N_9O_{16}$)

J.W. Quail, N. Ismail, M.S.C. Pedras, S.M. Boyetchko. 2002. Acta Cryst. C58:o268-o271

Compound	% Inhibition
ethyl acetate extract (0.6 mg/ml)	63 ± 6
Pseudophomin A (5×10^{-4} M)	67 ± 6
Pseudophomin B (5×10^{-4} M)	30 ± 4

Green foxtail in wheat with *Pseudomonas* as a pre-emergence application - 2006 field trials

Untreated **Low-rate** **High-rate**

Get the critical number/pop'n of BRG100 at the right place & time

How are the bacteria dispersed from the pesta?

Will crop competition enhance bioherbicidal potential?

Where do we place the granules?

Susan Boyetchko & Russell Hynes

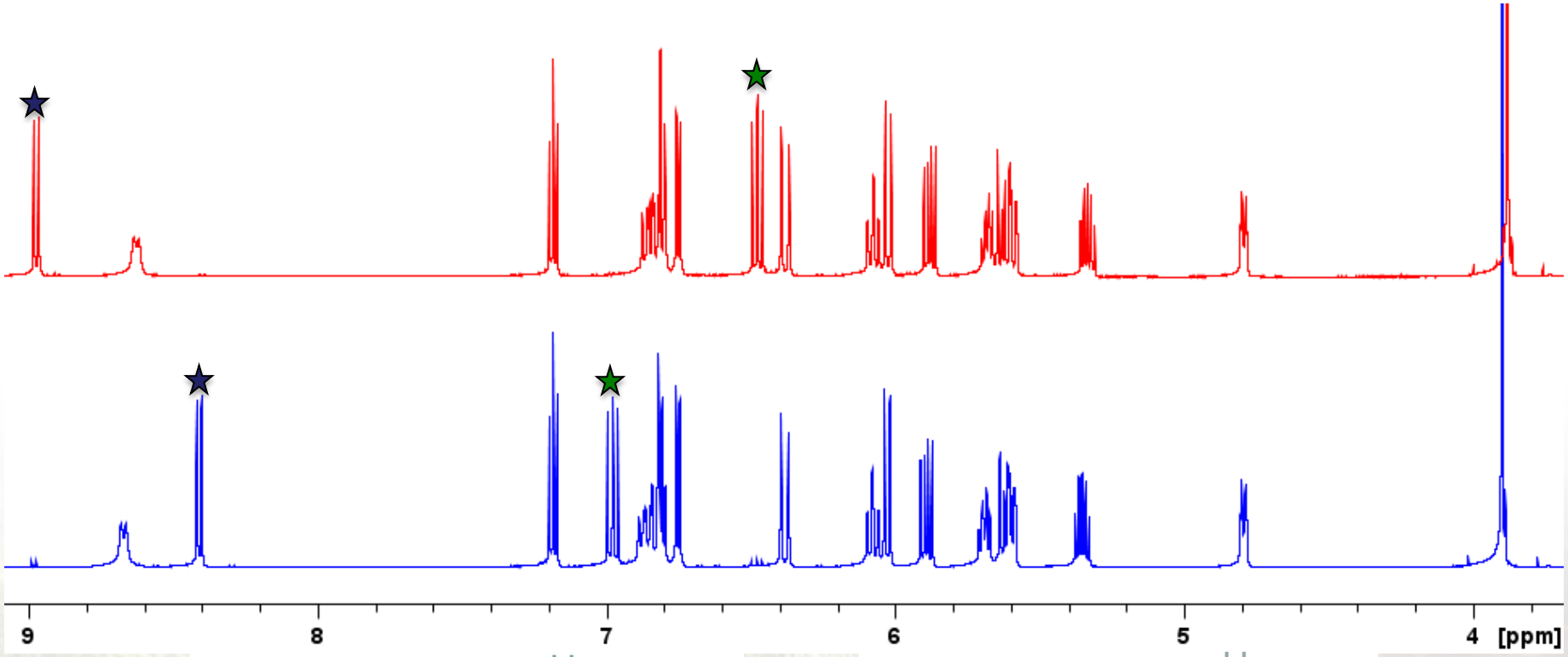
Potato Late Blight – *Phytophthora infestans*

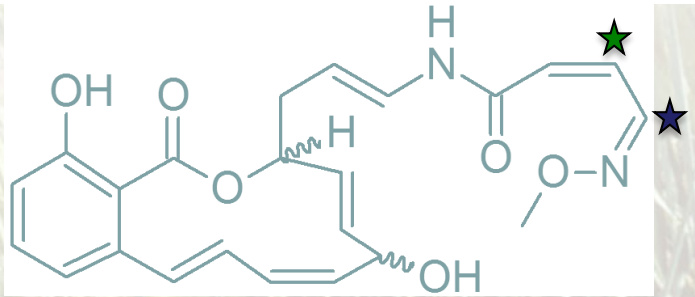
- Multi-billion dollar industry worldwide
- Seeded potatoes in Canada – 373,000 acres (2012)
- In Canada, potato production represents a market value of $1 billion; larger market of $5.5 billion for food processors
- Annual crop losses & cost of effective control measures ~ $6.7 billion globally
- Caused Irish potato famine in 1840s
- Multidisiplinary team (plant pathology, chemistry, microbiology, genomics, fermentation, formulation)
- Two 3 year projects
- **Filed PCT patent**
- **Industry partner – agreement with option to**
- **license**

Why should we pursue biopesticides for late blight?

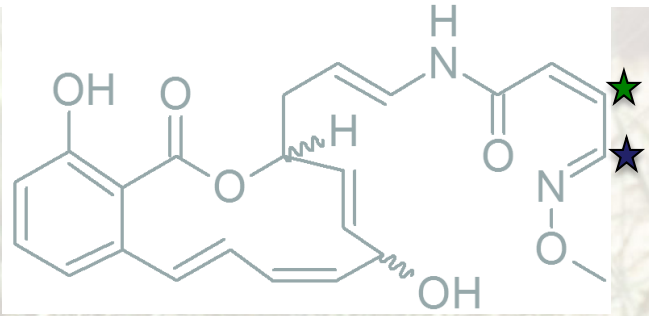
- New genotypes of the pathogen in Canada and the U.S. (US-8 ➔US-23); CA09
- Fungicide-insensitive isolates (metalaxyl, mefenoxam, etc)
- Multiple fungicide applications per growing season
  - Up to 12-15 applications per season
  - Cost millions per fungicide application
- Chemical pesticide load in environment
- Food safety and quality

^{1}H NMR of 189B vs. 189C



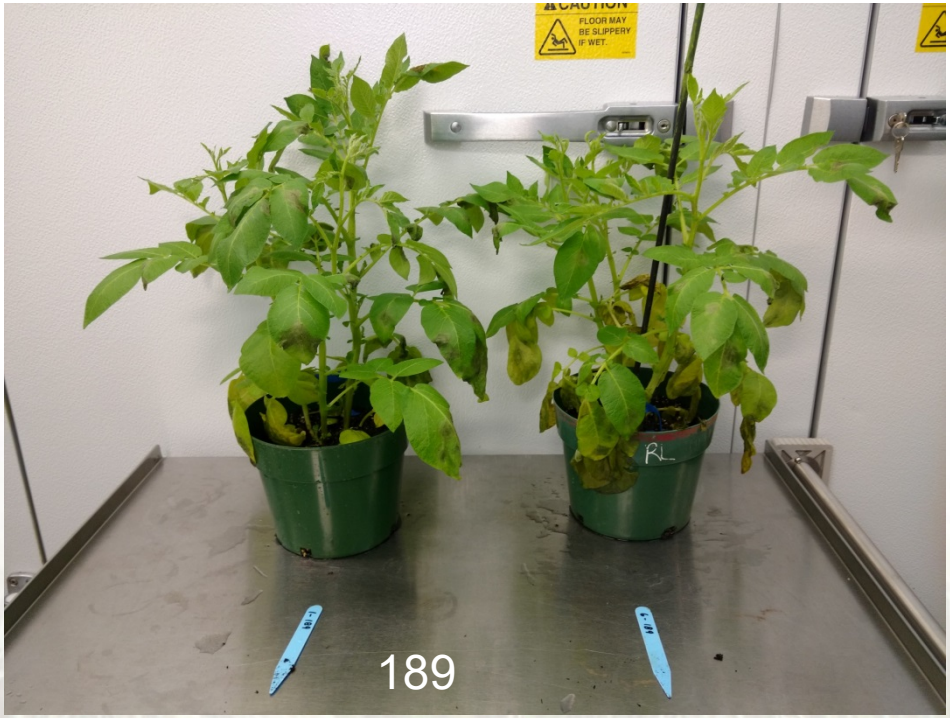


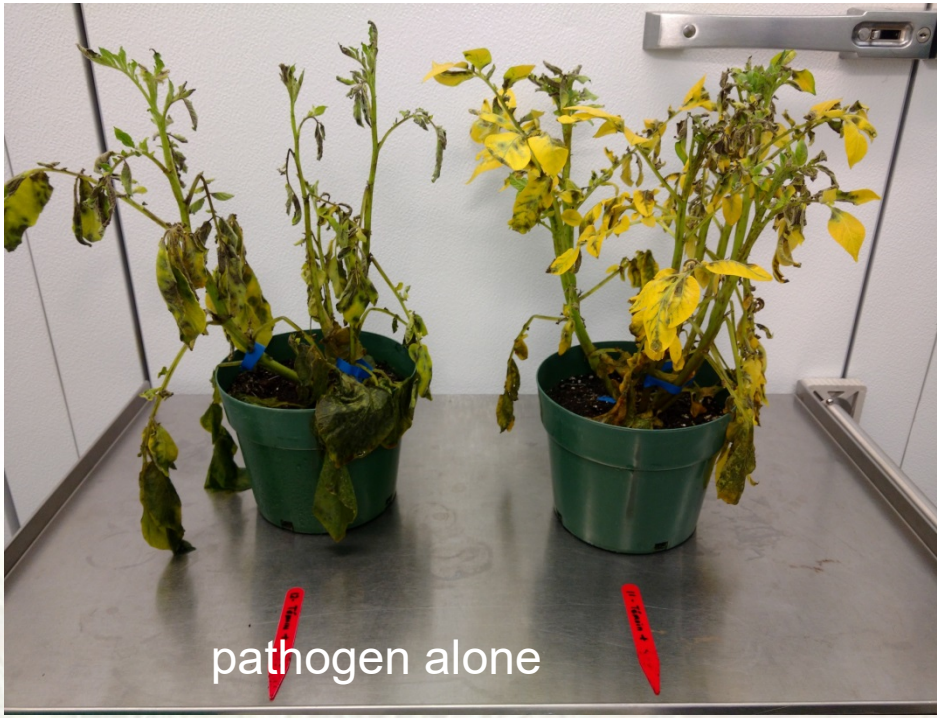
189B



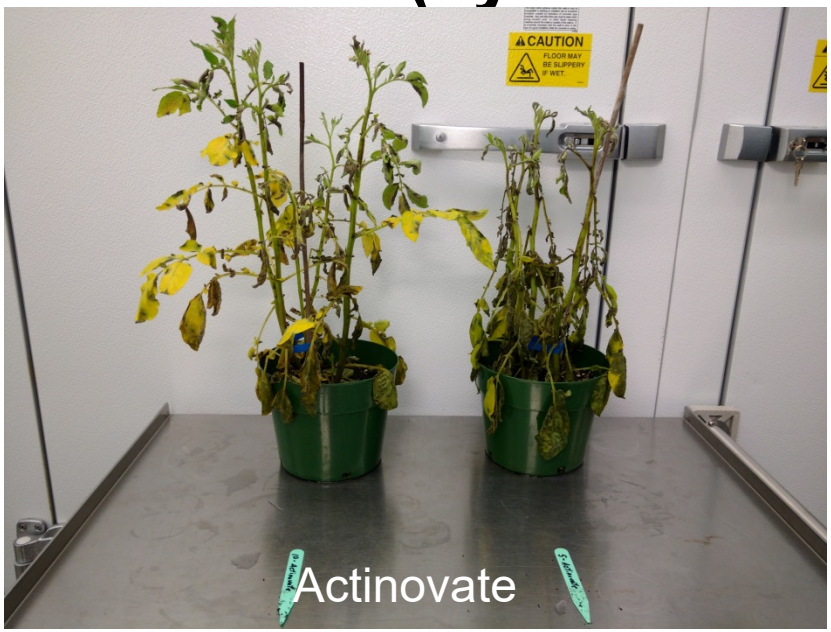
189C

Control of *Phytopthora infestans* with *Pseudomonas chlororaphis* strain 189





Comparison of efficacy with other control methods (synthetic fungicide & biopesticides)



Sclerotinia sclerotiorum in canola

- Canola is a multi-billion dollar industry in Canada
- Yield losses of 5-10%; up to 90% in 2010
- Full resistance to *Sclerotinia sclerotiorum* in canola remains elusive
- Synthetic fungicides show only single-site activity
- Chance of resistance to chemical fungicides
- Investigate green alternatives to chemicals as a clean technology for the canola industry

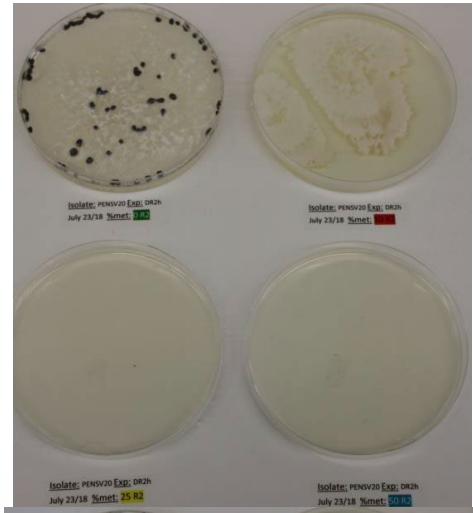
Objectives

- Screen and evaluate biopesticides (*in vitro, in vivo*)
- Conduct molecular characterization of the biopesticide candidate to improve its performance
- Understand the biopesticide mode of action (antifungal compounds, direct effect)
- Understand plant defense mechanisms
- Evaluate efficacy (does it work?)

Effect of Bacterial strain on *Sclerotinia sclerotiorum*

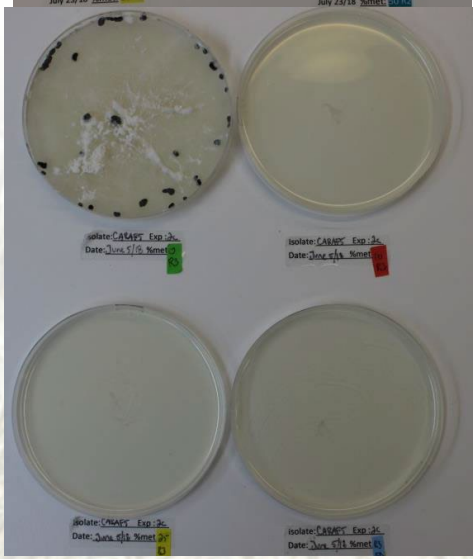
Bacterial strain PENSV20

Treatment (% CFCF)	Ascospore Germination	# of sclerotia	Sclerotial weight (g)
0	100 a	41.3 a	0.009 a
10	0.0 b	0.0 b	-
25	0.0 b	0.0 b	-
50	0.0 b	0.0 b	-



Bacterial strain CARAF5

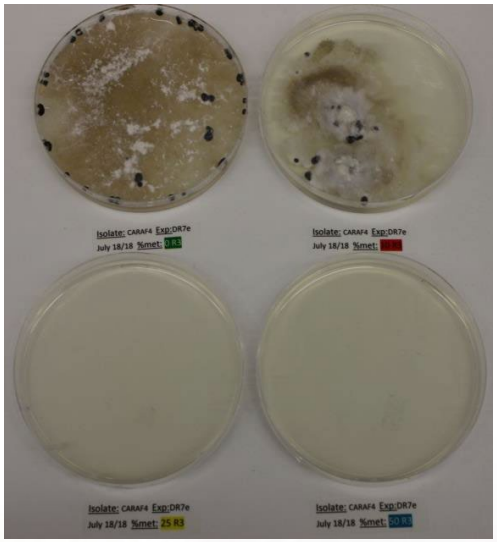
Treatment (% CFCF)	Ascospore Germination	# of sclerotia	Sclerotial weight (g)
0	100 a	39.8a	0.010 a
10	0.0 b	0.0 b	-
25	0.0 b	0.0 b	-
50	0.0 b	0.0 b	-



Effect of Bacterial strain on *Sclerotinia sclerotiorum*

Bacterial strain CARAF4

Treatment (% CFCF)	Ascospore Germination	# of sclerotia	Sclerotial weight (g)
0	100 a	26.3 a	0.011 a
10	0.0 b	13.3 bc	0.009 a
25	0.0 b	0.0 c	-
50	0.0 b	0.0 c	-



Bacterial strain YGM broth

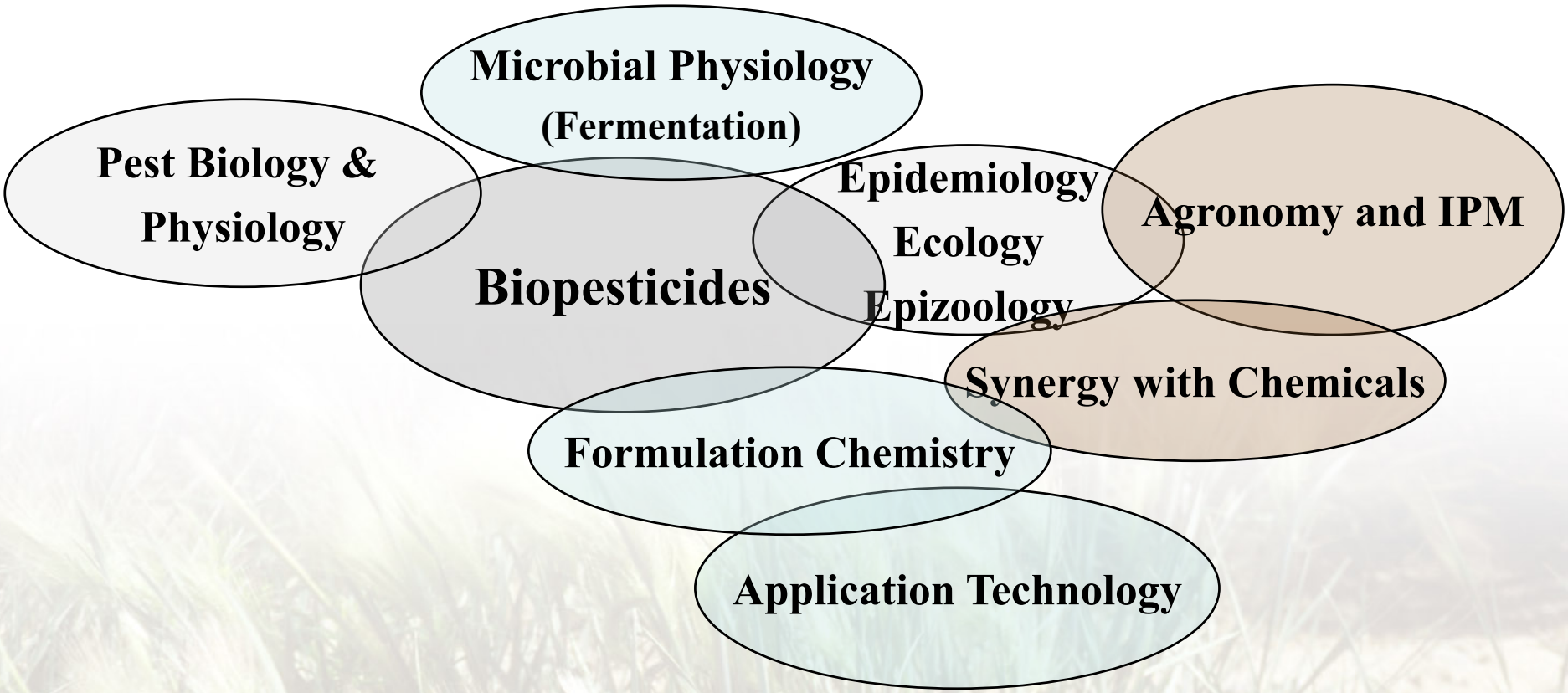
Treatment (% CFCF)	Ascospore Germination	# of sclerotia	Sclerotial weight (g)
0	100 a	38.2 a	0.010 a
10	95.8 a	36.7 a	0.012 a
25	99.2 a	36.7 a	0.011 a
50	101.4 a	39.0 a	0.013 a

Understand plant defense mechanisms

- Westar and Topaz (susceptible)
- Zhongyou 821 (partial resistance)
- Treatments: pathogen alone, BCA 24 h before pathogen, BCA 24 h after pathogen, sterile water
- Canola plants sprayed at 30% flowering stage with ascospore suspension
- RNA sequencing
- Canadian Light Source Synchrotron

Biopesticide for Sclerotinia

Multi-level biological systems require multidisciplinary approaches



Use of Integrated Pest Management tools will lead to successful adoption of biopesticides

So what is the future of biopesticides?

“If it was easy, everyone would be doing it”

- finding a microbial agent is “easy”
- discovering a “potential” biological control agent with all the desirable characteristics is much harder